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PROTEOMIC OF HUMAN NORMAL ARTICULAR CHONDROCYTES

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Introduction: Mitochondrias are involved in many cellular processes and mitochondrial dysfunctions have been associated with apoptosis, aging and number of pathological conditions, including osteoarthritis (OA). Mitochondrial proteins are an attractive target to study the metabolism of chondrocyte and its role in the cartilage degradation. However, contaminating proteins has been the major problem in all the mitochondrial proteomic research.
Aim: To obtain a Mitochondrial 2-DE proteome map of the human normal articular chondrocyte. To study whether purification of mitochondrias with density gradient ultracentrifugation using percoll and separation of proteins by 2D-E were useful to analysis the mitochondrial proteins of primary human normal articular chondrocytes
Material and Methods: Cells were isolated from five cartilages collected from autopsies without history of joint disease. Cultured cells were used to obtain different protein extracts (chondrocytes: total chondrocyte extract (CE); cytosol-enriched fraction (CY); crude mitochondria fraction (CM) and purified mitochondria fraction (PM)) which were resolved by 2-DE and visualized by silver nitrate or Coomassie blue staining. Spots were excised from the gels and analyzed using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or MALDI-TOF/TOF mass spectrometry.
Results: Results showed that purification of mitochondrias with density gradient ultracentrifugation using percoll and separation of proteins by 2D-E were useful to analysis the mitochondrial proteins of primary human normal articular chondrocytes. We identified a total of 62 unambiguous proteins from 90 excised spots (69%) by means of peptide mass fingerprinting (PMF). The spots were excised from each of three 2D-E gels carrying proteins from the PM fraction. Further 4 samples that could not be recognized with the information available in the PMF were identified by tandem mass spectrometry using MALDI-TOF/TOF technology. All together, our analysis leads to the identification of 66 spots that represent 45 different proteins. Analysis of proteins showed that 32% were located in the mitochondria, 15% in endoplasmic reticulum and 24% in the cytoplasm. According the biological function 21% are involved in protein targeting, 11% in signalling, 11% in glycolisys, 11% RNA/DNA protein synthesis, 11% in OXPHOS and 4% in redox. During aging, the purified mitochondrial fraction of chondrocytes showed increases in antioxidative proteins such as SOMD.
Conclusion: Because this technique allows the simultaneous identification and quantification of mitochondrial proteins in chondrocytes, it may help to determine alterations in protein levels that occur in cartilage degradation associated with OA.

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DECREASED TYPE II COLLAGEN PRODUCTION BY TRANSGLUTAMINASE INHIBITION IN PORCINE ARTICULAR CHONDROCYTES

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Aim of Study: Transglutaminases in articular cartilage ECM are responsible for retention and activation of latent TGFβ, and have been implicated in promotion of chondrocyte hypertrophy and CPPD crystal formation. TGases have been localized to the PCM of both normal and aging/OA chondrocytes, yet the role of TGase in the formation of healthy cartilage matrix and the organization of the chondrocyte pericellular matrix is not well understood.
Methods: Porcine articular cartilage was harvested from commercially obtained knee joints. Enzymatically isolated chondrocytes were grown in monolayer culture or in alginate beads in serum supplemented media containing monodansylcadaverine (MDC), or SY2011, a small competitive peptide inhibitor (PVKG). GAGs and type II collagen in collected media and GuHCl extracts were measured by DMMB assay or ELISA after 7 days. Cell viability was measured by MTT assay. Alginate bead cultures were aldehyde-fixed and dissolved after 3 days and chondrocytes were cast in thin agarose films. PCM were examined by immunocytochemistry using antibodies for types II and VI collagen and fibronectin.
Results: There was no change in GAG accumulation in media or cell-associated matrix by MDC at concentrations up to 50 μM (p=0.48; n=5). A small decrease (25%; p=0.05) was seen at 100 μM. GAGs were unaffected by the peptide inhibitor SY2011 up to 75 μM. In contrast, concentration-dependent decreases in type II collagen were observed with both inhibitors. MDC reduced total collagen accumulation by 21 and 27% at 12.5 and 25 μM, respectively (p=0.017, 0.002), and at 100 μM type II collagen was reduced by 65% (p=0.03). SY2011 lowered type II collagen production by 26% at 18.75 μM (p=0.02) and 47% at 75 μM (p=0.001) in a single experiment (4 replicates per group). Neither MDC nor SY2011 shifted the distribution of GAGs or type II collagen from cell associated matrix to culture media. MDC decreased cell viability only 6-10% ($p \sim 0.03$); SY2011 had no effect at 18.75 and 37.5 μM ($p > 0.1$) but reduced viability by 12% at 75μM (p=0.006). Chondrocytes had little or no ECM staining immediately after isolation. Some type VI collagen and fibronectin staining was evident within 24 hours, and after three days, chondrocytes had elaborated a PCM rich in type VI and type II collagen. Fibronectin staining was mostly limited to the cell surface. MDC and SY2011, at their highest concentrations, did not inhibit the deposition of collagens in the PCM, and cell death was not apparent.
Conclusions: Transglutaminase activity plays a significant part in the production of extracellular matrix by normal articular chondrocytes; however, despite the reported co-localization of tissue transglutaminase with pericellular matrix molecules, a role for TGase in the organization/retention of the PCM is not supported by these results.

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β-AMINOPROPIONITRILE TO STIMULATE COLLAGEN MATRIX REPAIR

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Background: Osteoarthritis is characterized by the progressive destruction of articular cartilage. Damage to the collagen network, one of the first signs of OA, is often considered irreversible, whereas proteoglycan loss is reversible. However, it was recently demonstrated that adult human cartilage does possess the capacity to synthesize collagen and incorporate it correctly into the existing matrix. In addition, treatment of cartilage transplants with collagen-degrading enzymes, greatly enhances the integration into the surrounding recipient tissue, indicating effective anabolic processes. These results open the possibility for the development of novel OA therapies directed at the modulation of collagen network formation. To fully appreciate the potential of this approach

it is crucial to better understand the processes that direct the formation of a functional collagen network in cartilage. The present study was designed to test whether initial blockage of collagen crosslinking would stimulate collagen synthesis and whether subsequent formation of the crosslinks would result in a functional collagen network.
Methods: Bovine articular chondrocytes were cultured in alginate beads for 5 weeks in the presence or absence of the lysyl oxidase inhibitor BAPN (0.1mM, 0.25mM or 0.5mM) followed by 5 weeks without BAPN. Proteoglycan synthesis (colorimetric assay), collagen synthesis and amout of crosslinks were measured biochemically (HPLC) at several timepoints.
Results: Inhibition of lysyl oxidase with BAPN caused an increase in total collagen levels. As from week 5, there was approximately 1.5 times more collagen deposition in cultures supplemented with BAPN than in the control condition, reaching 1.84 times more after 10 weeks (8.9 μg collagen/bead vs. 16.4 μg/bead). There was no difference in the proteoglycan deposition. Until week 6 there was a low amount of crosslinks in the collagen detectable in the matrix produced by chondrocytes in the presence of BAPN (0.18 pmol HP/pmol collagen) whereas collagen deposited without the presence of BAPN had a higher level of crosslinking (0.53 pmol HP/pmol collagen). From week 8 on, 3 weeks after removal of BAPN, the amount of crosslinks in all three conditions approached the level of the control condition (0.70 HP/collagen in the control condition vs. 0.60 HP/collagen with BAPN) in week 10. No dosis effect was observed for BAPN.
Conclusion: Blockage of collagen crosslinking with BAPN at first stimulates collagen synthesis compared to the control condition, followed by the formation of an equal amount of crosslinks after removal of the lysyl oxidase inhibitor. The effect of more collagen with the same amount of crosslinks per collagen molecule on mechanical properties requires further investigation.

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RAPID DEVELOPMENT OF OSTEOARTHRITIS IN MICE WITH OSTEOGENESIS IMPERFECTA

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The goal of this study is to demonstrate the role of the subchondral bone in the development and progression of hyaline cartilage degeneration in the knee joint.
We utilized a murine model of Osteogenesis Imperfecta (OI) to determine whether abnormalities in bone predispose the joint to the development of osteoarthritis.
OI is a genetic disease of humans that is caused by a mutation in type I collagen and is expressed in bone and ligament, but not in hyaline articular cartilage. The animals employed were a knock-in model of type IV OI and age matched wild-type (WT) controls.
High resolution micro-computed tomography (micro-CT) and micro-magnetic resonance imaging (micro-MRI) with gadolinium contrast enhancement, as well as histologic evaluation, were employed to study the knee joints of a pair of OI and a pair of WT animals age 2, 4, 6, and 12 months and one animal each at 22 months.
The results document for the first time that OI mice develop rapidly progressive OA. Micro-CT and micro-MRI documented age-related joint space narrowing, extra-articular ossification, and progressive subchondral bone thickening and cyst formation in the OI mice. Histologic examination showed typical aggressive cartilage destruction with osteophyte formation in the OI mice.
These results clearly demonstrate that abnormalities in subchondral bone can lead to rapidly progressive OA in the absence of intrinsic cartilage abnormalities.
Moreover, the data suggest that OI mice might serve as a useful model of OA that can be employed to examine the pathophysiology of cartilage damage as well as the impact of therapies.

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MATRIX METALLOPROTEASE ACTIVITY BUT NOT CYSTEINE PROTEASE ACTIVITY IS ESSENTIAL FOR ARTICULAR CARTILAGE DEGRADATION

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Objective: Both matrix metalloprotease (MMP) activity and cathepsin K activity have been implicated in cartilage turnover. We investigated the relative contribution of MMP activity and cathepsin K activity in cartilage degradation using *ex vivo* and *in vivo* models.
Methods: Bovine articular cartilage explants were cultured in presence of oncostatin M (OSM) 10 ng/ml, tumor necrosis factor-a (TNF-α) 20 ng/ml in the presence or absence of the broad-spectrum MMP activity inhibitor GM6001 and the cysteine protease inhibitor E64. Cartilage degradation was evaluated in the conditioned medium by measurement of crosslinked C-telopeptide fragments of type II collagen (CTX-II) and glycosaminoglycans (GAG), which were compared to immunohistochemical evaluations of proteoglycans and CTX-II. We assessed MMP expression by gelatine zymography and cathepsin K expression by immunohistochemistry.
Results: OSM and TNF-α in combination induced a 500-fold increase in cartilage degradation products measured by CTX-II ($P<0.001$) compared to vehicle control. The growth factors potently induced MMP expression, assessed by zymography, and cathepsin K expression investigated by immunohistochemistry. Inhibition of MMP activity by GM6001 completely abrogated CTX-II release ($P<0.001$) and inhibited proteoglycan release by 72% ($p<0.001$). In contrast, E64 resulted in an increased CTX-II release by 100% ($P<0.05$) and inhibited GAG release by 33% ($p<0.01$). An up-regulation of CTX-II fragments was confirmed *in vivo* in cathepsin K null mice.
Conclusions: Inhibition of MMP activity reduced both proteoglycan-loss and type II collagen degradation. In contrast, inhibition of cysteine proteases resulted in an increase rather than a decrease in collagen type II degradation, suggesting compensation by other proteases.

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IMPLICATION OF MITOCHONDRIAL DYSFUNCTION ON DEVELOPMENT OF INFLAMMATORY RESPONSE IN HUMAN CHONDROCYTES

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Aim of study: In this study we investigated if the inhibition of MRC is able to modulate the inflammatory response in human OA chondrocytes.
Methods: Human OA chondrocytes were isolated from cartilage obtained from the femoral heads of patients undergoing joint replacement surgery. Rotenone, 3-nitropropionic acid (NPA), antimycin A, sodium azide and oligomycin were employed to inhibit complex I, II, III, IV or V of CRM, respectively. LPS was employed as positive control. Cell viability was evaluated using the